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Recent Advances in the Study of Interferon

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I. Introduction

IN 1957, Isaacs and Lindenmann (43) discovered a soluble substance that they thought explained an obscure laboratory phenomenon called "viral interference." This is the ability of one virus to interfere with or reduce the replication of another virus. They named the substance interferon.

The basic elements in its production are still the same. An *inducer*, in their case inactivated influenza virus, acts on cells, in their case fragments of chick chorioallantoic membrane, to produce "*interferon*" demonstrable in the suspending medium. Interferon may then be used to *act* on cells to produce an antiviral state in the cells. This is demonstrated by challenging treated and control cells with an identical dose of virus. The treated cells replicate much less virus than the control cells. The minimal dilution at which a suspension containing interferon will inhibit viral replication is the basis of the *assay* of interferon.

More than 25 years of research have produced great advances in our understanding of what interferon is, the mechanism of its induction, how it acts, the scope of its biological activity, and its potential clinical applications. I can touch only briefly on each of these topics, with emphasis on the biological and clinical aspects of its use.

What is Interferon?

For many years, scientists doubted the existence of interferon. When I first studied it in human amnion monolayers infected with an attenuated type 2 poliovirus in the laboratory of Dr. J. F. Enders in 1958, he named it VIF, which stood for "virus inhibitory fluid" (36). This term was meant to be noncommittal. It could mean we were dealing with a negative factor, such as the absence of substances essential for viral replication. We discussed the possibility that we were dealing with the absence of some nutritional factor essential for cells. Later it was clear we were dealing with a protein. We thought we were dealing with a substance other than the interferon described by Isaacs and Lindenmann (43), because the chick cell interferon we got from Isaacs did not inhibit viruses in our human cell system. This was, of course a mistake. We now know that our VIF was *human interferon*, which unlike chick interferon, was active only in human cells.

There has been difficulty in purifying this substance. Now we know that interferon is really like other polypeptide hormones that are cellular products of very high specific activity but of which it is difficult to accumulate a large mass. The specific activity of interferon has been estimated to be about 4×10^{-9} mg protein per unit (or 2.5×10^{8} units per mg) (64). In an ordinary production system we are dealing with thousands or at best millions of units. For example, one unit of blood (500 ml) can produce 1 million units, or about 4 μg of interferon. This means that it is very difficult to get interferon in milligram quantities, which is needed for proper purification and characterization. Cells do not produce much interferon in terms of mass. This accounts for the difficulty of purification and also the difficulty of raising antibodies against interferon until 1962 (60), and the present difficulty in obtaining adequate amounts for clinical trials. Recombinant interferon, however, has already put an end to the shortage (see below).

As already intimated, interferon is not one protein but a class of proteins. There are several levels of heterogeneity:

1. Each species produces its own species-specific interferon. Human cells are needed to produce human interferon. This requirement is another factor limiting mass production of interferon.

2. Interferon varies depending on the type of cell producing it. Human leucocyte (α) interferon has been shown to be different from human fibroblast (β) interferon. Partial amino acid sequences from the NH_2-terminus of the 18,500 molecular weight species of lymphoblast and fibroblast interferon have been recently described (79, 48). These sequences are clearly different, although there are some similarities. The two polypeptides are also antigenically distinct.

3. The type of interferon produced also varies according to the method of induction. Species so far tested seem to have at least two types in this regard. One type is induced by inducers such as viruses, polyribonucleotides, endotoxin, etc. This is the type that has been purified and studied most extensively, such as human leucocyte and fibroblast interferon. Another type, γ or "immune" or "type II" interferon, is usually labile at pH 2. Many, including the immune interferon of man, are also heat labile. This type of interferon is the result of what may

be called "immune induction." It is produced when sensitized B or T lymphocytes are exposed to specific antigen. For example, immune interferon is produced when lymphocytes from pure protein derivative (PPD) or tuberculin positive individuals are incubated with PPD in vitro (21). Interferon is analogous to a lymphokine in this case, and is obviously an important effector substance in cellular immunity.

Inducers and Induction of Interferon

Viruses were the first inducers discovered. They are still one of the best. For example, human α interferon is made by exposure of buffy coat from units of donated blood to Sendai virus. An important discovery was that double-stranded polyribonucleotides are potent inducers (17). The most popular one is polyriboinosinic and polyribocytidylic acid copolymer, which can be made synthetically. The basis of interferon induction by viruses may be double-stranded RNA. Some viruses contain double-stranded RNA. In the case of others, this type of RNA may be made in the course of viral replication. This point is still disputed.

However, there are many other types of inducers that cannot be explained on the basis of nucleic acid, such as polysaccharides and microorganisms. They may act on macrophages to induce interferon by a different pathway.

All evidence supports the idea that interferon is a repressed cellular protein that is normally not made constitutively in the cell unless it is induced. Interferon induction is different from induction of enzymes in that it is a "one shot affair." Interferon production does not continue despite the constant presence of an inducer. Production is inhibited by inhibitors of protein synthesis, such as cycloheximide, and inhibitors of mRNA synthesis, such as actinomycin. Through use of these antimetabolites, it is possible to ascertain the critical period of induction.

The next important step in the study of the process of induction was the finding that when these antimetabolites were applied at proper times, they could paradoxically accentuate interferon induction. This phenomenon is similar to the phenomenon of superinduction described by Tomkins et al. (72) in the induction of tyrosine aminotransferase. It is explained by the inhibition of an inhibitor or repressor of interferon synthesis. The repressor is probably a protein that is also induced in the course of interferon production. Hence, it can be inhibited by substances like cycloheximide and actinomycin. The ability of these antimetabolites to enhance interferon production has been used to facilitate production of fibroblast interferon in fibroblast cultures (39).

It is now possible to isolate interferon mRNA from producing cells. Such mRNA can be made to translate interferon in cells of other species, such as *Xenopus* oocytes (5).

Cloning of Interferons

A recent advance in the study of interferons is the successful production of both α (leucocyte) and β (fibroblast) human interferons in plasmid-transformed *Escherichia coli*. Nagata et al. (58) collected 5000 clones, and one of these, "Hif-2h," produced 2×10^4 units per liter of *E. coli* culture. Similar results were obtained by Goeddel et al. (20) (see table 1). The DNA sequence of one such clone (pL 31) codes for an interferon peptide of 165 amino acids preceded by a 23-amino-acid signal peptide. About 2.5×10^8 units of interferon per liter could be produced. One of the most interesting aspects of these studies is that by analyzing the nucleotide and amino acid sequences, closely related but not identical types of α-interferons were discovered. These also have varying biological properties. For example the host range of these interferons differ (table 2). The Genetech group have identified A, B, C, D, E, F, and W variants. Nagata's clone probably is the same as W.

Nagata, Mantei, and Weissmann (57) used a human gene bank consisting of a collection of hybrid phage and fetal human chromosomal DNA from an embryo. These were screened by ^{32}P-labelled interferon-α1 cDNA probe. Sixteen hybridization positive phage clones were isolated from 240,000 plaques. No less than eight unique chromosomal nucleotide sequences were isolated. The chromosomal genes for interferon-α were also unique for lack of introns.

The cloning of β-interferon was reported by Derynck et al. (11). In this case, the interferon gene was inserted in a thermoinducible expression plasmid under control of the phage lambda P_L promotor. Processing to a size compatible with mature but unglycosylated β-interferon

TABLE 1
Cloning of α-interferon in Escherichia coli (20)

1. mRNA (poly (A) RNA) is collected from KG-1 (human myeloblast) cells induced with Sendai virus.
2. cDNA prepared from mRNA by reverse transcriptase.
3. cDNA tailed with deoxyC residues annealed with plasmid pBR322.
4. *E. coli* K12 strain 294 transformed by plasmid which have tetracycline and ampicillin resistance markers.
5. Positive clones were those whose plasmid DNA hybridized with interferon cDNA (30 out of 500).
6. One of these produced LeIFA (pL 31).

TABLE 2
Host range of cloned α-interferon

Cell Line	Species	Cloned Interferon			Natural α-Interferon
		A	B	F	
WISH*	Man	100	100	100	100
Vero	Monkey	250	250	1400	400
BHK	Hamster	400	2	0	50
RK-13	Rabbit	12	15	0	120
L	Mouse	150	16	500	0

From N. Stebbing, Genetech.
* 100 signifies 100% of base-line antiviral effect with VSV as challenge virus.

was observed. It is expected that γ-interferon will also be cloned. With cloning, rapid advances in the understanding of the structure, genetic determination and regulation of these molecules are expected.

As of this writing (October 1981), a little over a year after the cloning of α-interferon, phase 1 and phase 2 studies of recombinant interferon in man are already partially completed. This must be one of the most rapid applications of basic research to medicine. The following tentative conclusions may be made: 1) The problem of shortage of supply has probably been solved. 2) The biological effects of the cloned material, including its side effects, are quite similar to leucocyte interferon.

Mechanism of Action of Interferon

The main features of how interferon acts were realized quite soon after its discovery. We showed that interferon had no direct effect on the virus, nor did it inhibit its adsorption on the cell (36). Interferon acted on the virus-infected cell rather than the virus. It inhibited the transcriptive or translational ability of viral nucleic acid. This was demonstrated when chick interferon-treated chick cells inhibited the replication of infective poliovirus RNA (34).

Another important discovery was that the action of interferon required the synthesis of an intermediary protein (71). The action of interferon was inhibited by inhibitors of mRNA and protein such as actinomycin and cycloheximide. The identity of the so-called Taylor protein is still unsettled, but recent studies show that there are many candidates.

I think it is safe to say that we still do not understand the details of how interferon works. The conclusions vary somewhat with the cell-virus system, and new phenomena have been discovered as one looks at the problem with different techniques and concepts. The most important action seems to be inhibition of the translation of protein synthesis. There are at least two novel mechanisms explaining this action that have occupied workers recently.

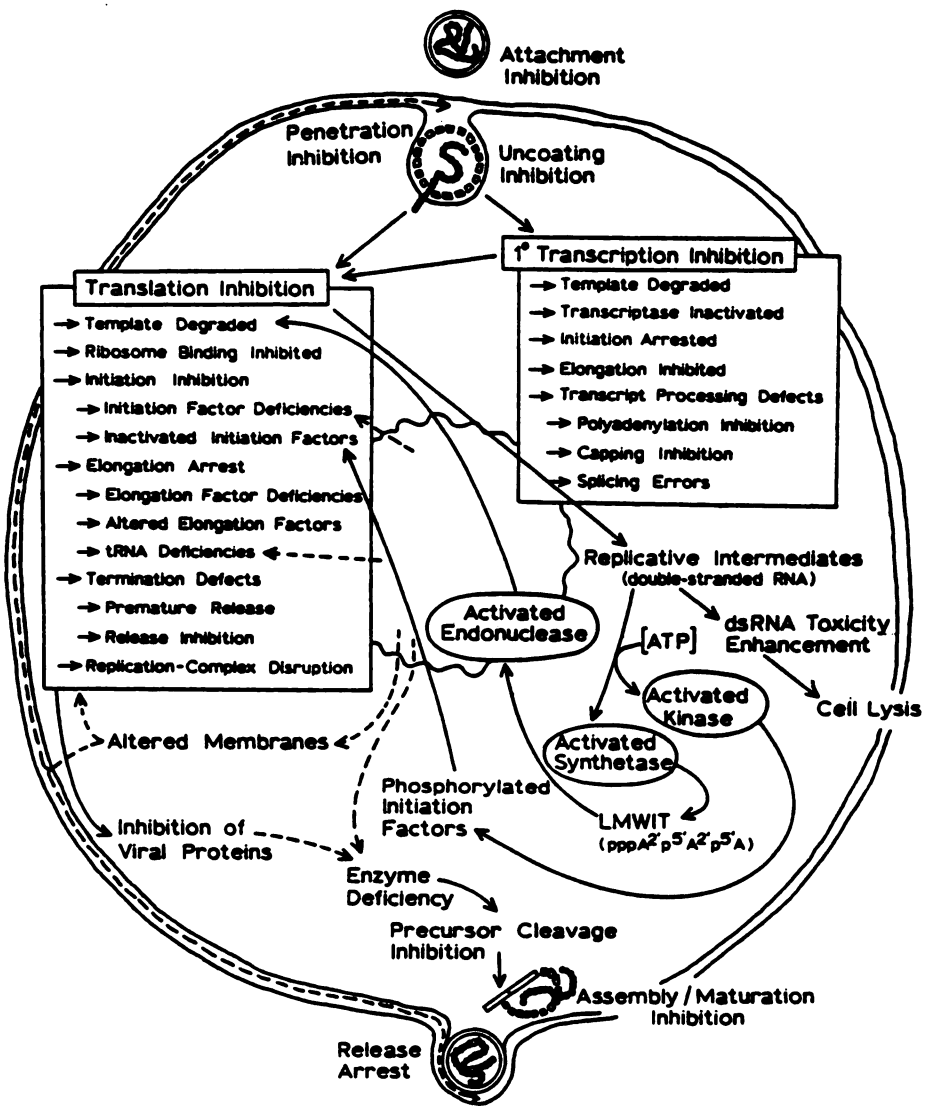

FIG. 1. The many antiviral actions of interferon. (By W. E. Stewart II, The Interferon System, Springer-Verlag, New York, 1979.)

Kerr and Brown (46) discovered an enzyme in interferon-treated cells that synthesized a new class of oligomers of 2′,5′-isoadenylate, which have novel 2′,5′-phosphodiester linkages between the riboses of the adenylic acid instead of the usual 3′,5′ linkages. This new class of molecules can specifically activate an intracellular ribonuclease F, which degrades mRNA, and hence inhibits protein synthesis, including translation of viral proteins.

Another candidate for Taylor protein is protein kinase. Protein kinases have now been found in interferon-treated cells that phosphorylate eIF-2, one of the initiation factors of protein translation. Phosphorylation inactivates the initiation factor, and thereby inhibits protein synthesis, including viral protein synthesis (49).

Actually there are many other parameters of antiviral activity that have been studied. These include inhibition in interferon treated and infected cells of transcription (formation of viral mRNA), elongation arrest of viral peptides by lack of certain tRNAs, and inhibition of release, particularly in oncornavirus infected cells (18). In the case of Moloney leukemia virus, interferon favors accumulation of a presumably uncleaved glycoprotein. Perhaps synthesis of a cleaving enzyme is blocked (6). A simplified summary is presented in Figure 1.

Interferon's Action and Biological Scope

For the first 10 years after the discovery of interferon, it was thought to be solely a unique antiviral with remarkable specificity against viral synthesis. The early work indicating that interferon may also inhibit multiplication of cells was widely disbelieved and thought to represent the effect of impurities. Due largely to the persistent efforts of I. Gresser, it is now generally accepted that interferon can directly suppress the growth of certain tumors, apart from its effect on viruses (25). This fact accounts for the widespread interest in interferon as an antitumor agent.

Paucker et al. (61) first showed that interferon could inhibit cell multiplication, an observation that is now generally accepted after a period of confusion and uncertainty. Interferon does not kill cells, nor does it inhibit all of them, whether normal or malignant. Its effect may be minimal and it is reversible. Some cells, such as Daudi cells, may be inhibited by as little as 1 unit, and others may be completely resistant (1). During inhibition, it does not block any particular phase of the cell cycle but slows progression of cells through all phases. Creasey et al. (8) studied the effect of human interferon-α on human melanoma cell lines. Cells in a quiescent stage at high density ("A" or G_0-G1 state) were more sensitive to the effect of interferon than those that were in the "B" phase consisting of the S and G2 + M state. Perhaps interferon influences a step representing commitment to DNA synthesis. Adding interferon once DNA synthesis has begun, during the "S" phase of the cell cycle, produces minimal inhibition.

Nissen et al. (59) reported interferon suppressed colony formation by bone marrow cells. Verma et al. (74) maintained that the plating efficiency was not affected by interferon. It reduced the number of colonies formed in agar clonal assays but increased the number of "clusters" with fewer than 40 cells. Most of these were immature granulocyte precursors. Presumably interferon causes leucopenia, one of its most important side effects (see below), by blocking myeloid differentiation.

A number of other activities have been discovered that together demonstrate that the antiviral action of interferon is but one of many (table 3). Earlier we pointed out that interferon is produced as a lymphokine when sensitized lymphocytes are exposed to specific antigen or even nonspecific mitogens. Interferon has now been shown to have many effects on immunological reactions.

The paradoxical effects of interferon on immunity may be illustrated by its effect on antibody synthesis and delayed hypersensitivity. Braun and Levy (3) found that an intraperitoneal injection of a low (250 to 1,500 units) dose in mice enhanced production of antibody to sheep red blood cells while a high dose (5,000 to 10,000 units) inhibited production. The effect of interferon on delayed hypersensitivity was related to timing rather than dose (10). Interferon administered 24 hours before the antigen inhibited subsequent sensitization. When given a few hours after the antigen, sensitization was enhanced. In a clever experiment with mice that were either low or high interferon producers, DeMaeyer and his wife (10) showed that interferon actually played a role in the expression of hypersensitivity. Newcastle disease virus (NDV), a sensitizing antigen as well as interferon inducer, was injected in C_{57}BL/6 or Balb/c mice, which are respectively the high and low producers. A sensitizing dose of NDV also produced interferon several hours after injection. When these mice were challenged with NDV in the footpad 4 days later, more footpad swelling was produced in the C_{57}BL/6 mice than similarly treated Balb/c mice. Anti-interferon serum given at time of sensitization eliminated the difference in the reaction. These results show that interferon induced by the antigen enhanced the hypersensitivity reaction.

Interferon reduces the graft versus host reaction (33). Verma et al. (74) thought interferon may impede successful bone marrow grafting because of its antiprolifer-

TABLE 3
Many effects of interferon

1. Antiviral Effects
2. Cell suppressive effect: Effect on tumors
3. Modulation of humoral and cellular immunity (antibody synthesis and delayed hypersensitivity)
4. Enhancement of phagocytosis, activation of macrophages
5. Stimulation of natural killer cells (also antibody dependent cell-mediated cytotoxicity)
6. Cell surface alterations: Enhancement of surface antigens (H-2), Enhancement of toxicity (poly I:C), Resistance to toxin (diphtheria)
7. Effect on interferon production: Priming, Blocking

ative effect, but such untoward effect was not observed in a recent trial of interferon in bone marrow recipients suffering from cytomegalovirus infection (56).

Interferon affects surfaces of cell membranes, an action that may underlie some of its immunological effects. Gresser observed rounding of human amnion cells after treatment with interferon (24). Pfeffer et al. (63) found that the cell volume, protein and DNA content, and attachment surface in normal and tumor cells were all increased by fibroblast (β) interferon. Interferon increases the resistance of cultured cells to diphtheria toxin perhaps by interfering with its uptake (77). It interferes with the uptake of thyrotropin and cholera toxin (47). Thyrotropin added to cells before interferon also prevented the development of the antiviral state; this suggests competition of the two substances at receptor sites.

Incubation of mouse lymphocytes with interferon increased the expression of H-2 surface antigens but not theta or Ia antigens (50, 75). This effect may underlie the enhancement of T cell cytotoxicity in mixed lymphocyte reaction. There is some controversy as to which killer lymphocytes can be enhanced by interferon. Trichieri and Santoli (73) do not believe interferon can augment the killing by killer (K) cells in antibody dependent cell-mediated cytotoxicity (ADCC), while Herberman et al. (31) do. They believe all effector cells which have Fc receptors can be augmented by interferon. These are natural killer (NK) cells, monocytes, and a subpopulation of E-rosette forming T cells that have Fc receptors and mediate lectin-induced cell-mediated cytotoxicity. Alloimmune cytotoxic T cells are resistant to interferon.

There is no disagreement that a very important effect of interferon is the stimulation of the NK lymphocytes (19, 33, 73). The NK cell is a non-B, non-T lymphocyte with Fc receptors on its surface, which is able to kill nonspecifically tumor cells and virus-infected cells. It is thought to be an important defense mechanism against proliferation of tumor cells in the body. NK cells may also be important in defense against virus infections. They are stimulated by interferon formed 1 to 3 days after infection, at a time before specific immunity becomes operative (76).

Unlike a number of other immunological effects of interferon, the stimulation of NK cells is exquisitely sensitive to small doses of interferon. Interferon may well be one of the primary regulators of NK activity in the body. The basis of its action is still unclear, although RNA and protein synthesis but not DNA synthesis are required (32). We have shown recently that lymphocytes obtained from a patient after prolonged interferon therapy may also be depressed by interferon (39a). The significance of this observation is still unclear.

Interferon can activate macrophages and enhance phagocytosis (40, 41), another possibly important immunity-enhancing effect. The mechanisms are unknown, but it may be a surface phenomenon.

Interferon is not only a lymphokine produced by sensitized lymphocytes in the presence of specific antigen, it is also produced by lymphocytes after stimulation by nonspecific mitogens. Both α (or β) as well as γ interferons may be produced. The best example is the production of acid stable, non-γ mouse interferon in mice after the injection of lipopolysaccharides (LPS) (78). Interferon produced by macrophage cell cultures stimulated by LPS is also of the β type (51). The terms type II and "immune" interferon are misleading and should be abandoned. γ-Interferon is differentiated from other types on the basis of acid lability and antigenicity (42).

There is some evidence that interferon is produced by concanavalin A-activated suppressor cells (45). Suppressor activity was abolished by anti-interferon, which suggests interferon mediates suppressor activity. The significance of this observation requires confirmation.

The cell source of γ-interferons is also a confusing subject. Some of the questions will not be resolved until the various interferons produced are adequately characterized. Falcoff et al. (15) seem to equate immune interferon with T cell interferon. Epstein et al. (14) have shown clearly that interferon may be produced by both B and T lymphocytes stimulated either by concanavalin A or pokeweed mitogen.

γ-Interferon may not be equivalent to other types in terms of biological activity. It has greater antitumor effect than β-interferon against sarcoma MC-36 in mice (65). It was 2000 times more potent than β-interferon in inhibiting the number of antibody-producing splenocytes of sensitized mice. As little as 2.4 units of γ-interferon significantly reduced the number of cells producing antibodies against sheep red blood cells in a Jerne assay (66). This is one type of interferon that has not been purified to homogeneity or cloned in bacteria.

Biological Significance of Interferon

Since interferon is a natural product of cells, it has always been assumed to play a role in host defense mechanisms against virus. Experiments to prove this were conducted in Gresser's laboratory (27) with mice infected with encephalomyocarditis virus. The effect of interferon produced naturally during the infection was neutralized by the administration of antiserum against interferon. The infection was much more severe in such mice showing that the interferon formed had a protective effect.

How interferon functions naturally in other respects is less clear. For example, we know nothing about the maintenance of NK cell levels in normal animals. How is interferon involved? How is it involved as an immunomodulator normally? If so, how important is it? These questions still remain to be answered.

The interferon response is probably part of the primitive immunological apparatus. Congenital deficiency of interferon response has not been described. It may indeed be incompatible with life.

Pharmacokinetics of Interferon

Precise studies on the pharmacokinetics of interferon have not been numerous for a number of reasons: (1) Pure interferon has not been available in adequate quantities, nor has a satisfactorily labeled compound been prepared. The lack of these makes it difficult to study its fate in various body compartments. (2) The biological assay for interferon has a large inherent error, and is inadequate for measuring small differences. (3) Interferon is a protein without any identifiable metabolic split products. There are essentially no data on its degradation and metabolism.

There is some confusion about the meaning of blood and tissue levels of interferon. Our concepts about the meaning of blood levels, at least for those of us interested in infections, come from the study of antibiotics, agents that directly affect the target of their action. Sustained concentration level is translated as high antimicrobial efficacy. No such translation can be assumed in the case of interferon. It does not act directly on viruses, but only indirectly by making cells resistant to viruses. Since this effect takes time to bring about, cells need not be exposed to high interferon levels at the time of virus infection, but some hours before.

Ordinarily, blood levels of an antibiotic are a reflection of tissue levels, and hence availability. But in the case of a sizable protein whose absorption and metabolism is unknown, this may not be the case. Interferon in the blood stream is essentially excluded from many body compartments. It has been suggested for example that the reason why blood levels of fibroblast or β-interferon are low is because it is absorbed in tissues (13). This would mean, paradoxically, that the lower the blood level, the better the therapeutic effect. Unfortunately, data are inadequate to clarify this and the other issues indicated.

Interferon behaves in the vascular compartment very much like a protein whose molecular size is around 20,000 daltons. It is not impermeably contained in the compartment like larger proteins, such as albumins or globulins, nor does it permeate freely through the endothelium like smaller polypeptides. We determined the half-life of rabbit interferon in the rabbit to be 11 minutes after an intravenous injection (38). Similar values have been obtained for mouse and human α interferons. This means that interferon levels cannot be long sustained in the bloodstream unless it is constantly reintroduced. The decline of serum concentration with time after an intravenous bolus injection presents a complex curve with many components. The first part of the curve, first order and exponential in nature, has been used to measure a serum "half-life." Less effort has been made to treat in a quantitative fashion the meaning and significance of the subsequent less steep components of the disappearance curve. The reason is that to obtain reliable constants, large amounts of interferon and a precise method of titration are needed. Rabbit interferon may be found for several days in serum despite an extremely rapid initial decrease.

To study the pharmacokinetics of interferon, the "two compartment" model may be considered (23). The first is the "central compartment" consisting of the blood, extracellular space, and highly perfused tissues and organs including the kidney, which excretes interferon irreversibly. The second or the "peripheral compartment" consists of less well perfused tissues, such as muscle, skin, body fat, and the central nervous system into which interferon enters more slowly. Interferon enters and is eliminated via the central compartment, but there is reversible transfer between the central and peripheral spaces.

The initial rapid fall in concentration, observed in all interferon elimination curves, may be called the "alpha" distribution phase, where α is the slope. The second, slower phase is the "beta phase" where β is the slope. α and β may be measured graphically in min^{-1} or $hour^{-1}$. The half life of interferon during the α and β elimination phases may then be calculated:

$$T_{1/2}\,\alpha = \frac{\ln 2}{\alpha} = \frac{0.693}{\alpha}$$

$$T_{1/2}\,\beta = \frac{\ln 2}{\beta} = \frac{0.693}{\beta}$$

For any drug $T_{1/2\beta}$ is the important statistic, and not $T_{1/2\alpha}$, for $T_{1/2\beta}$ measures the degree of exposure of tissues to interferon [see Greenblatt and Koch-Weser (23) for further details]. These constants have been determined by one of our doctoral candidates for rabbit interferon (16).

Absorption into the vascular system is slower after an intramuscular injection, and detectable levels may be obtained for a longer period of time. A dose of 5 million units of α interferon a day produces a constant, measurable serum interferon level of about 30 units/ml (62). In contrast, doses of β-interferon up to 20 million units produced no serum levels (2). McPherson and Tan (52) found only 4 to 12 units of β-interferon in the serum 4 hours after the injection of 10×10^6 units, and none before or after. It is possible that β-interferon is more avidly bound by tissues and more rapidly cleared from the bloodstream because of higher glycosylation.

Interferon from the vascular compartment enters with difficulty the cerebrospinal space, brain, placenta, fetus, and aqueous and vitreous humor. The reverse is in most cases also true (67). DeClercq et al. (9) injected 6×10^5 units of α-interferon intrathecally one to two times a day in an infant with herpes encephalitis. Twenty-four hours later, the titer in the spinal fluid was 0.1% to 1.3% of the initial dose, and the amount in the serum was 40- to 80-fold less. Similar results had been obtained by Ho et al. (35) in rabbits.

Renal excretion of interferon has been studied only in

rabbits (37). Only about 0.2% to 2.0% of interferon was excreted, a figure that has not been reproduced in man. We induced serum interferon with NDV in the rabbit and used the conventional renal clearance formula UV/Pt, where U = interferon concentration collected over time t, V = volume of urine, t = minutes or hours of collection, and P = mean plasma interferon concentration over time t. In the rabbit, serum NDV-induced interferon was cleared by the kidney at the rate of 30.6 ml of plasma per kg of rabbit weight per hour. This represented about 25% of the hourly glomerular filtrate.

Comparable excretion data are not available in man or other species. After injection of 20×10^6 units in a human subject, no detectable interferon was recovered in the concentrate of urine collected over 24 hours (unpublished data). In the same subject, we maintained a relatively constant plasma interferon concentration over 2 hours by a constant intravenous infusion of 4.8×10^6 units. Urine collected over this time period had no detectable interferon. It is possible that human interferon is less well excreted than rabbit interferon. This cannot be explained by differences in molecular size (the rabbit molecule is larger, 46,000 daltons), but it may be due to differences in degradation or tissue uptake. One hypothesis is that the kidney pools and degrades, but does not excrete, interferon.

Toxicology of Interferon

Perhaps the most frequent side effect, also seen with fibroblast interferon (2), is fever. In our experience, fever occurred in some patients after 2.5 to 5.0 million units of partially purified human leucocyte interferon despite concomitant administration of corticosteroids in high doses (62). Elevations in body temperature were tolerable even when doses as high as 5×10^5 units/kg/day were given (55) and decreased after three or four doses (53). We found that after a lapse of 1 week, tolerance to the febrile response was lost. Purer preparation of both α and β interferons have produced less fever. It is doubtful that it can be entirely eliminated. There are no studies on the mechanism of fever production.

Reversible suppression of white cells, reticulocytes, and platelets have been observed 3 to 5 days after initiation of high doses of α interferon therapy in lymphoma patients with herpes zoster (55). Doses of 8.5×10^4 units/kg/day or greater over longer periods depressed these cells in patients with chronic active hepatitis, but not in patients with normal livers; this suggests that liver dysfunction or prolonged administration may enhance bone marrow-related side effects with interferon (22). Cheeseman et al. (7) noted that 4.3×10^4 units/kg intramuscularly every other day in immunosuppressed renal transplant patients caused transient leucopenia and thrombocytopenia and the schedule had to be reduced to twice weekly. Billiau et al. (2) described a transitory (<8 hours) leucopenia in patients receiving fibroblast interferon. No instance of irreversible bone marrow suppression has been reported after interferon treatment. Recently, as much as 200 million units of cloned α_1 interferon were injected in a single dose in cancer patients without any irreversible suppression (Gutterman and co-workers, Second International Conference on Interferon, San Francisco, October 21–23, 1981).

The mechanism of transient bone marrow depression is not understood. It does not appear to be related to recruitment of cells from the bone marrow, or sequestration of leucocytes. As mentioned above, perhaps it is best explained by the decrease of colony-forming ability of marrow cells in the presence of interferon (59), which is probably a manifestation of the anticell proliferation effect, one of the basic biological properties of interferon. There are fewer data on the effect of β-interferon on leucocytes. Billiau et al. (2) saw lymphopenia. McPherson and Tan (52) report no leucopenia with 10×10^6 units of partially purified material.

Mild to moderate hepatic dysfunction has followed prolonged administration of interferon. There is almost always a mild elevation of liver enzymes, such as SGOT (62), which is of no clinical consequence. Lassitude and malaise observed with chronic administration of interferon limited ambulatory dosage to 1.7×10^5 units/kg/day or less in Merigan's experience (53). These effects may be accentuated in cancer patients. Gutterman et al. (29) observed more fatigue, anorexia, and weight loss in cancer patients over 70 years old. Minimal alopecia occurred in 16% of patients. Some patients developed herpes labialis, presumably as a result of the pyrexia. There were also complaints of dry mouth and dry eyes.

Some patients who received fibroblast interferon had precipitating antibody to bovine serum, a component of the medium used in making the interferon. Immediate type of cutaneous allergy was also observed in some patients (2).

In view of the many immunological effects of interferon, one might expect profound disturbances of the immune system as an undesirable side effect. Except for effects on leucocytes, this has not been the case. No opportunistic viral or bacterial infections have been described. Strander et al. (69) described a drop in the complement fixation titer against a number of virus antigens and mycoplasma in 10 out of 11 patients. In patients who received greater than 10^5 units/kg of α-interferon per day, Hafkin et al. (30) observed a decrease in the lymphocyte transformation against herpes simplex and varicella-zoster viruses, but not at lower doses. We observed no change in the lymphocyte proliferative response to phytohemagglutinin (PHA), concanavalin A, or herpes simplex antigen in a patient who received 148×10^6 units in 16 weeks (unpublished data).

As more interferon becomes available for clinical trials, and as we probe the upper dosage limits, note should be taken of some striking results in mice. Gresser et al. (28) injected C243 mouse cell interferon of a specific activity of 0.3 to 3×10^6 units/mg of protein titering 1:800,000

into neonatal mice. All of them died within 9 to 14 days with degenerated livers after receiving an estimated daily dose of 6.4×10^6 standard units/kg. Injections in older mice or a 10-fold smaller dose was innocuous. If neonatal mice were injected for only 6 to 8 days, the liver damage was reversible, but the mice developed progressive immune complex glomerulonephritis (26). Whether these changes are peculiar to mice, or are limited to neonates, or related only to the large doses used is unknown.

As mentioned above, genetic engineering has probably solved the problem of interferon supply. Many types of pure recombinant interferon are now being tested in patients. So far, cloned α_1 interferon produced in patients fever, transient lymphopenia, lassitude, and other side effects described for leucocyte interferon. In a few patients, antibodies against this cloned material were produced, while antibodies against impure natural leucocyte interferon have not been produced in extensive clinical experience with this material (Gutterman and co-workers, Second International Conference on Interferon, San Francisco, October 21–23, 1981). Whether this reaction is a function of dose, or the administration of a subspecies of interferon essentially foreign to human subjects is not known. One cannot a priori expect a "pure" product to behave better than an impure, natural one.

Clinical Application of Interferon (Virus Infections)

Interferon is relatively nontoxic, and is broadly active against many viruses. It has been seriously considered as an antiviral agent since it was discovered. I understand that in Eastern European countries, particularly the Soviet Union, interferon has been sold over the counter for respiratory infections for years. Its effect has been more assumed than proven. Real progress requires the accumulation of solid evidence, which has been slow, and is often undramatic.

Experience with interferon illustrates the importance of controlled studies to demonstrate therapeutic efficacy. With a scarce material, and in a situation when effects are not dramatic, carefully controlled, preferably blind placebo controlled studies, are essential. Controlled studies are expensive, time consuming, and occasionally not possible to undertake. The results may be marginal, but they are conclusive.

It has been shown in controlled studies that interferon is effective in the following human clinical conditions:

1. Prevention and amelioration of the symptoms of the common cold following intranasal inoculation of rhinovirus type 4 (54).

2. Reduction of the acute symptoms and possibly the number of recurrences in herpes simplex keratitis (44).

3. In doses up to 20 million units per day, interferon reduced the morbidity and spread of herpes zoster in patients with lymphoma (55).

4. Interferon reduced viremia and symptoms of cytomegalovirus in renal transplant recipients (7).

5. Interferon reduced the activation of herpes labialis given before the reactivation event by our group (62). More details will be given below.

Interferon has been shown in uncontrolled studies to reduce the antigen load in patients with chronic active hepatitis (22). Core antigen and DNA polymerase, which are components of the virus circulating in the bloodstream of such patients, are reduced. These studies are exciting because if true, they indicate that interferon is effective in a chronic DNA virus infection. It is, however, not known that the patient's basic pathological condition is improved by the treatment. One disturbing feature is that it is very difficult, if not impossible, to cure the patient's antigenemia (HB_sAg) despite months of treatment.

We had a rather unusual opportunity to study the effect of interferon on herpes simplex infection (62). An operation to relieve trigeminal neuralgia was devised by our chief neurosurgeon at the University of Pittsburgh, Dr. Peter Jannetta. By using microsurgery, he dissects off small arterioles that constrict the trigeminal root. This "decompression" operation produces instant relief that lasts for varying periods of time. The trigeminal ganglion is the site of latency of herpes simplex type 1 infection. It has been known since Harvey Cushing performed a periganglionic neurectomy to relieve trigeminal neuralgia, that operation on the ganglion is a potent stimulus for the reactivation of herpes labialis (4). We found that 65% to 80% of patients who had a history of recurrent herpes labialis will develop cold sores 2 to 3 days after the Jannetta operation even though that operation, unlike the old Cushing operation, does not involve cutting any nerve fibers. What we had was an unusual collection of patients who predictably developed a virus infection within a few days of a definite inciting event. We decided to test whether 7×10^4 units per kg per day of interferon given for 5 days beginning the evening before the operation could prevent virus reactivation in a double-blind controlled study. The patients were followed for 1 to 2 weeks after surgery. In 18 patients who received a placebo, oral virus shedding occurred in 15. In 19 patients treated with interferon, virus shedding occurred in eight. The intensity of virus shedding was greatly reduced by interferon. Of 127 daily throat wash cultures collected from the placebo group, 42% were positive, while only 9% of 134 cultures collected from the treated group were positive. These results are highly significant. This study shows that interferon given at the proper time can prevent reactivation of herpes virus infection. Studies are in progress to determine whether interferon is effective when given before or after surgery. Preliminary results already indicate that 10.5×10^4 units/kg given 1 day before surgery in three divided doses is completely effective. Studies are continuing to determine whether interferon given immediately after surgery is effective (unpublished results).

These clinical studies taken together show that inter-

feron, to be effective must be given in millions (1 to 20) of units per day for at least 4 or 5 days. In no situation has interferon been found to be curative.

The amount required for achieving a modest clinical effect precludes immediate clinical application of interferon. This problem may be overcome if interferon becomes as cheap and plentiful as, for example, penicillin. Interferon may turn out to be important as a preventive drug. It may eventually be used as an adjuvant to other forms of therapy. In any case, I do not believe its precise place in clinical medicine has yet been defined. Certainly the many biological actions of interferon suggest we are dealing with an extremely complex substance, which, unlike penicillin, may have profound, unforeseen effects on the host. Interferon has certainly opened new vistas in the biomedical sciences, if not yet in medical therapeutics.

Clinical Application of Interferon (Cancer)

The history of anticancer therapy is punctuated by exaggerated hopes and disappointments. No doubt exaggerated hopes were vested in interferon. Whether it will turn out to be a complete disappointment remains to be seen.

No controlled studies of interferon as therapy for cancer have yet been done. However there have been enough uncontrolled trials so that the range of expectation should already be in focus. In a summary of clinical studies with interferon up to 1979, Dunnick and Galasso (12) listed trials against the following tumors: osteogenic sarcoma, multiple myeloma, juvenile laryngeal papilloma, cervical carcinoma, breast cancer, non-Hodgkin's lymphoma, melanoma, and condyloma acuminata. The result of each trial was alleged to be favorable.

Perhaps the most famous and influential of these was the treatment of osteogenic sarcoma with interferon by Hans Strander and his colleagues in Sweden (68). This is a type of malignancy of children that has a fairly predictable, uniformly fatal outcome. Patients received 3×10^6 units of interferon daily for 30 days, followed by 3×10^6 units three times weekly for 17 months (estimated total dose 7.5×10^8 units). Of 33 treated patients, 69% were alive at the end of 3 years and 61% were free of metastases. The comparable figures for 36 controls treated with conventional chemotheapy, consisting of methotrexate or adriamycin, were 34% and 37%. It should be noted that these were historical controls, and not simultaneous patient controls.

More recent trials have been conducted by Borden et al. (First International Congress on Interferon, Washington, D.C., 1980) and Gutterman et al. (29). These two groups treated 43 patients with breast cancer, who received 3 to 9 million units of interferon a day for 28 to 84 days. No patient achieved complete remission, 12 (55%) achieved partial remission (defined as some objective improvement and 50% decrease in measurable lesions), and six (14%) achieved "improvement" (defined as 25% to 50% decrease in measurable lesions). The remainder showed no remission. The disease of seven out of 26 of Borden's patients (27%) progressed.

Gutterman et al. (29) also observed some effect in six out of 10 patients with multiple myeloma (60%), and five out of 11 patients with malignant lymphomas (46%). One patient with multiple myeloma and two with lymphoma achieved complete remission.

The following conclusions may be ventured: 1) Probably as F. Rauscher announced, interferon is no magic bullet against cancer (70). 2) The results are no better, but probably no worse than other known types of therapy in a group of patients with advanced disease, many of whom had failed conventional therapy. 3) Improved dosing may improve results. 4) Interferon is a novel anticancer agent that may act synergistically in conjunction with other agents even if it is not a magic bullet when used alone. Forthcoming studies will undoubtedly answer these questions.

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